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THE NATURE OF THE $\overline{B}$–$\overline{A}$ TRANSITION OF DNA IN SOLUTION

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1. Introduction

As has already been shown by the early X-ray studies of double-stranded DNA [1], the conformation of this molecule depends on the relative pressure (P_{rel}) of water vapour with which a DNA sample is in equilibrium. (P_{rel} = relative humidity = $P_{H_2O}/P^o_{H_2O}$, where the numerator and denominator are water vapour pressures in the system and that above pure water at the same temperature, respectively). That form of DNA stable at $P_{rel} \leqslant 0.75$ was called the A form and that form stable at high P_{rel}, the B form. Further investigations showed that whether DNA exists in the A or B conformations is determined not only by P_{rel}, but also by the nature and quantity of the cations present in the sample [2]. The structures of both forms of DNA have been described in the work [3], where the latest refined atomic co-ordinates are given.

It is quite clear now that it is more correct to speak not of individual A and B conformations, but of $\overline{A}$ and $\overline{B}$ families of forms [4]. To the $\overline{B}$ family, along with the canonic B form belong the C [5], T and some other closely related forms [6] of DNA. A-DNA and the complementary RNA helices (A-11 and A-12) belong to the $\overline{A}$ family of conformations [7]. No discrete conformation within the families seem to exist in solution and a continuous transition due to a gradual change in the helix parameters is possible [4], whereas the interfamily transition both in fibrous samples [6] and in solution [4] occurs abruptly.

Development of a circular dichroism (CD) method made it possible to study the conformational transitions within the double-stranded state not only in fibers, but in solution as well [4,8,9].

Brahms and Mommaerts [10] found that the CD spectrum of DNA in 80% ethanol solution is similar to that of RNA. Thus, in such a solution DNA acquires a conformation belonging to the $\overline{A}$ family. The $\overline{B}$–$\overline{A}$ transition of DNA in water–nonelectrolyte solutions has been recently studied in our works [11,12].

As P_{rel} equals the value of water activity in a system ($a_{H_2O} = P_{H_2O}/P^o_{H_2O}$, providing that the non-ideality of water vapour is not taken into consideration), it is natural to pose the question of whether the belonging of the solution DNA conformation to the $\overline{A}$ or $\overline{B}$ families is controlled mainly by the value of water activity as it is in fibers and films, or the $\overline{B}$–$\overline{A}$ transition is essentially dependent upon the nature of the non-water components.

2. Results

The $\overline{B}$–$\overline{A}$ transition of calf thymus DNA was

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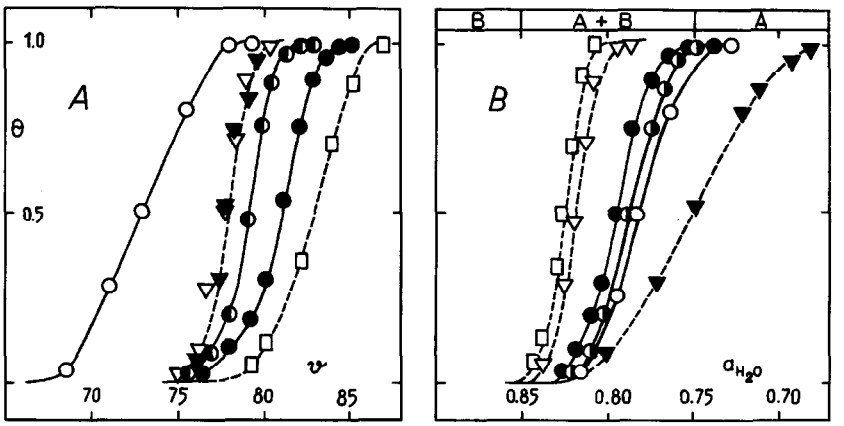

Fig.1. Curves of the $\overline{B}$–$\overline{A}$ transition as a function of the increase in the proportion of the non-electrolytes in water; the relative amount of the A form is $\Theta = \left(\frac{\Delta D - \Delta D^B}{\Delta D^A - \Delta D^B}\right)_{270}$ where ΔD^B and ΔD^A are the CD magnitudes at 270 nm for the $\overline{B}$ and $\overline{A}$ form respectively; ΔD is the magnitude in the transition region; (○) is for eth–water and (●) is for iso–water mixtures. The ternary systems were obtained by addition to a 75% iso–water solution (v/v) calculated quantities of methanol (▼), eth (●), dio (▽), and THF (□). The mixtures thus contained a variable content of non-electrolyte. Thymus Na–DNA was in concentrations from $5 \cdot 10^{-5}$ to 10^{-4} M (P); the NaCl concentration in the samples, as revealed by a flame photometer, was about $2 \cdot 10^{-4}$ M. The solutions were prepared as described in the paper [4]. A. The dependence of Θ on volume percent of the non-electrolytes. B. The dependence of Θ on water activity in the system (see table 1). At the top of fig.1B are indicated the regions of the existence (and coexistence) of the $\overline{B}$ and $\overline{A}$ conformations according to X-ray data [2,7] and the infra-red dichroism study of Pilet and Brahms [20].

studied by CD in the following systems: ethanol (eth)–water, isopropanol (iso)–water, isopropanol–dioxane (dio)–water, isopropanol–ethanol–water, isopropanol–methanol (meth)–water, and isopropanol–tetrahydrofurane (THF)–water. In all the systems mentioned the $\overline{B}$–$\overline{A}$ transition, brought about by an increase in the content of the non-polar component, did take place (fig.1). In the studies [4,8,12] it was shown that the observed change in the CD spectrum indeed corresponds to the $\overline{B}$–$\overline{A}$ transition (This point is specially discussed in [12]). In addition, no hyperchromicity was observed during the $\overline{B}$–$\overline{A}$ transition. Thus, the observed shift is entirely within the double-stranded state. A family of the CD spectra at the different proportions of alcohols is presented elsewhere [4,11,12].

In the case of the ternary systems the solutions were prepared in the following way. First DNA was dissolved in 75% (v/v) iso–water solution (The concentrations of DNA and counterions, indicated in the legend to fig.1, are essential for the successful dissolving with no precipitation). In this solution DNA still exists in the $\overline{B}$ form as revealed by CD. Then small amounts of the third component were stepwise added, the CD spectrum being recorded after each addition.

The water activity values for our 'cocktails' were calculated from the water pressure values taken from the handbook [13] and the review [14]. There were also used the water vapour pressure data for the iso–

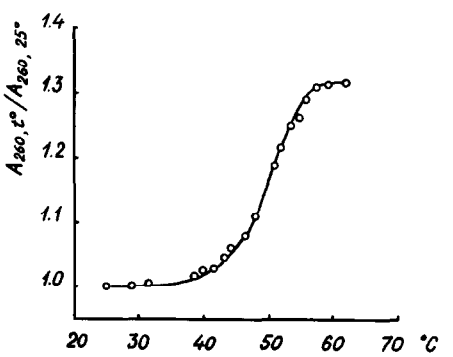

Fig.2. The two-stranded state of DNA in 80% ethanol as revealed by melting pattern. Calf thymus DNA (concentration 10^{-4} M in phosphates) was in 80% water-ethanolic solution with 5×10^{-4} M NaCl.

water [15] and iso–eth–water [16] systems. The activity coefficients for the THF–water system can be found in a recent study [17].

Interpolation of the a_{H_2O} values and calculation of those in the ternary systems were done using the 3-suffix Morgules equation, whose coefficients were taken from the review [14] or found from the experimental values by the least squares method. A modified White's equation was also used for the interpolation of the a_{H_2O} values for the binary (1) and ternary (2) systems:

$$\ln \gamma_2 = \left(\frac{ax_1}{bx_1 + x_2}\right)^2 \quad (1)$$

$$\ln \gamma_2 = \left(\frac{ax_1 + cx_3}{bx_1 + dx_3 + x_2}\right)^2 \quad (2)$$

Here $\gamma_2 = a_{H_2O}/X_2$ is the activity coefficient of water, X is the mole fraction of a corresponding component (the indexes 1 and 3 refer to non-electrolytes, 2 is for water); a, b, c, d are the empirical coefficients found by the least squares method from the experimental values of γ_2 for the concentration range studied.

Extrapolation of the a_{H_2O} values, calculated for elevated temperatures, to 25°C was done according to the equation:

$$\frac{d \ln \gamma_2}{d T} = -\frac{\triangle \overline{H}_2}{RT^2} \quad (3)$$

Where $\triangle \overline{H}_2$ is a partial enthalpy of water in the systems, R is the gas constant. $\triangle \overline{H}_2$ values were calculated from heat of mixing data, taken from the book [18]. As it was found that in the range of concentrations and temperatures, essential for our study, $\triangle H_2$ is nearly linear with temperature, equation (3) can be integrated in the following way:

$$\ln \gamma_2 = -\frac{H_0}{RT} - \frac{A}{R} \ln T + B \quad (4)$$

H_0 and A were determined from the dependence $\triangle H(T)$, B was obtained from γ_2 value for some temperature. All three parameters depend slightly (in our range) on concentration. The calculations were performed with a 'Nairi' computer. Values of a_{H_2O} calculated by different methods and using data of different authors agree within 2%. As the width of the $\overline{B}$–$\overline{A}$ transition is of the same order of magnitude, this occuracy is not sufficient to judge if the $\overline{B}$–$\overline{A}$ transition curves coincide for the different systems when plotted as a function of a_{H_2O}. The most precise data are those available for the binary systems eth–water and iso–water systems. For these systems the data of different studies coincide with each other

Table 1

Values of water activity, a_{H_2O}, in the systems eth–water, iso–water and in the ternary system iso–water + eth

Volume % of non-electrolyte	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
a_{H_2O} in eth–water system*	0.827	822	817	812	806	800	793	786	779	770	762	752	742	731	719	707						
a_{H_2O} in iso–water system**											0.830	827	823	820	813	805	800	790	775	764	750	738
a_{H_2O} in iso–eth–water system***											0.830	826	812	798	790	772	757	741	723			

* Data of [14], extrapolated to 25°C as described in the text.

** Data of [14], extrapolated to 25°C according to Morgules eq. These coincide with the data of [13] with the accuracy of 0.5%.

*** Calculated after White's eq. for a three-component system, using the data of [14] and [13].

within 1%, except the data of the work [15], which for the iso–water system leads to a_{H_2O} values, which are higher by 1.5% as compared with the data of [13,14] and [19].

The water activity values, used by us for plotting the $\overline{B}$–$\overline{A}$ transition curves (fig.1B), are given in table 1. Presence of inorganic ions and DNA was not taken into consideration in calculating a_{H_2O}, since the concentration of these components was very small and their influence on a_{H_2O} was less than the accuracy with which this value can be determined.

As can be seen from fig.1B, the $\overline{B}$–$\overline{A}$ transition in all the systems studied occurs when a_{H_2O} lies within the limits from 0.85 to 0.70 and half-transition points lie in the region 0.83–0.75. This region coincides with the range of the relative humidity ($\equiv a_{H_2O}$) values, at which the $\overline{B}$–$\overline{A}$ transition is observed in a DNA fibers (films)-water vapour system, studied by X-ray analysis [1–3,6] and by optical methods [8,20,21]. Thus, Brahms and Pilet [20,21] give 0.95 and 0.75 relative humidity values for the limits of the $\overline{B}$–$\overline{A}$ transition region (regardless of the source of DNA), with most of their curves lying within 0.85–0.75.

3. Discussion

Thus, the water activity (or chemical potential) values in solution, as well as in fibers and films, determine in which in the two types of conformations, $\overline{A}$ or $\overline{B}$, the DNA helix exists. This is quite natural, for these two classes of conformations are essentially different in the degree of base-pair stacking due to the different pattern of overlapping of the base pairs. In media with high water activity the hydrophobic interactions would stabilize the $\overline{B}$-like conformations with greater stacking [7]. In media with a low water activity the contribution of the hydrophobic effect is less, and DNA acquires the $\overline{A}$ conformation, which is more preferable from the point of view of non-valent interactions within the sugar-phosphate backbone [7].

Clearly, water activity, being a major factor which determines the type of DNA conformation, is not the only one. In solutions and fibers the transition point depends on inorganic and organic cations interacting with the phosphate groups of DNA. Interestingly, the width of the $\overline{B}$–$\overline{A}$ transition in fibers, determined by the X-ray method [7], and that in solution, determined by CD is practically the same (2–4%, a_{H_2O}). Thus, the interactions between the DNA molecules in fibers are not great enough to change the cooperative properties of the system, which are therefore determined by the intrinsic properties of the DNA molecule.

Last we wish to draw attention to the specific effects of the different non-water components. As can be seen in fig.1B, the curves for eth–water, iso–water and iso–eth–water cocktails are almost identical. (The same is true for the dio–water and THF–water mixtures). But the difference in the curves obtained for the meth containing system, on the one hand, and those for THF or dio, on the other hand, are rather well pronounced. This can be explained by supposing that the added non-electrolytes, especially methanol, have a hydrophobic effect, to some extent imitating water [4]. This supposition is supported by the fact that in meth–water solution [4] or in ethylene glycol [22] DNA is present in a conformation of the $\overline{B}$-type (C) at water activity much less than 0.75. (In fact it is not possible to transform DNA into the $\overline{A}$ form in the binary aqueous systems of these components). From the data of fig.1B the 'water-likeness' of the substances studied form the series: water > methanol > ethanol ⩾ isopropanol > dioxane ⩾ THF.

It is remarkable that in the same series the polarities of the substances diminish, the polarities of methanol (and ethylene glycol) not being low enough to evoke the $\overline{B}$–$\overline{A}$ transition in the binary system. This explains the difference between methanol and ethanol, which puzzled the authors of a recent paper [23] and made them search for an explanation on the basis of an aggregation phenomenon in ethanolic solution.

The conclusion of the descisive role of water activity in the $\overline{B}$–$\overline{A}$ transition could be biologically interesting, as in the cell there are the regions of low polarity (e.g. membranes), which make contact with the DNA of a chromosome.

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